Supporting Information

Materials and Methods

Plant materials

The rice cultivar Pokkali (IRGC 108921, *indica,* containing *BPH9*) was obtained from the International Rice Research Institute (Los Banos, the Philippines). Other rice germplasm used in this work were obtained from the National Rice Research Institute and the Institute of Crop Science, Chinese Academy of Agricultural Sciences. Pokkali and the susceptible *indica* variety 9311 were used as parental lines for constructing a *BPH9* mapping population. The Pokkali/9311 F_1 plants were backcrossed 7 times with 9311 through marker-assisted selection (MAS), and the near isogenic lines (NIL-*BPH9*) carrying *BPH9* gene in 9311 genetic background were selected from the BC_7F_2 population. The genotypes of NIL-*BPH9* were determined using molecular markers.

Brown planthoppers

BPH populations of biotypes 1, 2 and 3 were maintained on rice varieties TN1, Mudgo and ASD7, respectively, in a greenhouse at Wuhan University as previously described (1). Wild population of BPH insects were collected from rice fields in Wuhan, China, and maintained on susceptible cultivar TN1.

BPH resistance evaluations

BPH resistance of rice populations was evaluated at the seedling, tillering and mature stages using at least three replicates of each cultivar or line as previously described methods (2, 3).

Briefly, for the bulked seedling test, about 20 seeds were sown in a row of 15-cm length in a plastic box. At the third-leaf stage, the seedlings were infested with second to third-instar nymphs of BPH at eight insects per seedling. When all of the seedlings of susceptible control died, the plants were examined and each seedling was given a score of 0, 1, 3, 5, 7, or 9 as described by Huang (2). To evaluate resistance at tillering and mature stages, each of the rice plants was grown in a 20-cm diameter plastic bucket and infested with 100 adult BPH insects. About a month later, the plants were scored as susceptible (dead) or resistant (alive).

Performance of BPH insects on rice plants was evaluated as previously described (3). For the two-host choice, a susceptible and a resistant rice plants were grown in the same plastic cup. At the four-leaf stage, the cup was covered with nylon mesh and nymphs were released (10 second to third-instar nymphs per plant) in the cup.

Locations where the BPH insects had settled on each plant were recorded at 3, 6, 24, 48, 96 and 120 h after infestation. For BPH weight gain and honeydew excretion assay, newly emerged female adults were weighed and enclosed in a pre-weighed Parafilm sachet (each Parafilm sachet contain one female adult) and attached to the leaf sheath of the rice plant. After 48 h, the insects were removed from the sachets. Both the insects and the honeydew of each sachet were weighed again. The weight difference of BPH insect was recorded as weight gain of BPH, the weight difference of the Parafilm sachet was recorded as honeydew excretion. At least 30 BPH insects were used for analysis. Honeydew was stained with 0.1% ninhydrin in acetone solution (3). For ease of comparison, we choose Mudgo (containing *BPH1*) to represent the same allele type of *BPH1/9-1* including *BPH1/ BPH10/ BPH18/ BPH21*, and ASD7 (containing *BPH2*) to represent the same allele type of *BPH1/9-2* including *BPH2/BPH26*.

Map-based cloning of *BPH9*

To map the *BPH9* gene, 135 $F_{2:3}$ plants derived from a cross between 9311 and Pokkali were evaluated for their BPH resistance and subjected to SSR markers analysis. *BPH9* was initially mapped to a region on chromosome 12L flanked by molecular markers RM28486 and RM28438. To fine map *BPH9*, we used the flanking markers RM28486 and RM28438 to screen 3,000 BC_3F_2 plants and obtained 32 recombinants. Six InDel and SSR markers in the RM28486-RM28438 region were obtained and used for genotyping the recombinants. *BPH9* was delimited to the genomic region flanked by InD2 and RsaI. For further fine mapping, we used InD2 and RsaI to screen 10,000 BC_5F_2 plants and obtained 4 recombinants. New markers (Table S4) were developed using the 9311 and Pokkali genomic sequences. We also obtained markers from GRAMENE (http://www.gramene.org/markers/index.html). A fosmid library of Pokkali was constructed by Takara Bio (Takara, Dalian, China).

Complementation tests of *BPH9*

To construct the plasmids for the complementation test, a 15.6-kb genomic DNA fragment of *R2* (containing the promoter region, the entire CDS region and the downstream sequence), digested by *Eco*RI and *Sal*I from fosmid clone 544-22, and a 11.3-kb genomic DNA fragment of *R1*, digested by *Bam*HI from fosmid clone 19, were inserted into the binary vector pCAMBIA2300 and the modified pCAMBIA1301 by replacing GUS reporter gene with blue-green fluorescence (BGF) (4) respectively. The *R2* cDNA containing construct, including its promoter region,

the entire ORF and the 3' untranslated regions (UTR), were amplified and digested by *Sal*I-*Nco*I, *Nco*I-*Xho*I, *Xho*I-*Eco*RI, respectively, and then inserted into the modified pCAMBIA1301 (*SI Appendix*, Fig. S4). After verification by sequencing these constructs were transformed into the BPH-susceptible *indica* variety Kasalath using the *Agrobacterium*-mediated method (5). Plants regenerated from hygromycin-resistant calli (T_0 plants) were grown, and T_1 seeds were obtained after self-pollination. The genotypes of each transgenic plant and their progenies were examined with gene-specific primers and Southern blotting. The T_1 cDNA-transgenic plants and homozygous T_2 genomic-transgenic plants were used for BPH resistance test.

RNA isolation, quantitative RT-PCR analysis and RACE

Total RNAs were isolated from rice plants using TRIzol reagent (TaKaRa) and then converted into first-strand cDNA according to the manufacturer's instructions. Expression of *BPH9* and other genes involved in BPH feeding responses was analyzed by quantitative RT–PCR using SYBR Green PCR Master Mix (Applied Bio systems) and a CFX96 Real-Time System (Bio-Rad) following the manufacturer's instructions. Sequences of the primers used are listed in *SI Appendix*, Table S4. The results were analyzed using CFX Manager Software 2.1. *OsSDHA* and *OsTBP* were selected as internal controls. All results had three biological replicates and three technical replicates each containing a pool of 15 plants. The cDNA 5'-RACE and 3'-RACE sequences were obtained using a SMART RACE cDNA amplification kit (Clontech, Mountain View, CA, USA), following the manufacturer's instructions. Primers are listed in *SI Appendix*, Table 4.

Microarray analysis

The second or third-instar nymphs were transferred to 4-week-old seedlings of NIL-*BPH9* and 9311 plants (10 nymphs per plant) in a box covered with nylon mesh. Leaf sheaths of the rice plants infected by BPH were collected at 0, 3h and 6h after BPH infestation for microarray analysis, with each sample containing a pool of at least 100 plants. Total RNA was isolated using an RNAiso Plus kit (TaKaRa) following the manufacturer's instructions. The transcriptomic profiles of the leaf sheath samples were then obtained by hybridizing with the Affymetrix Rice Genome Array (GPL2025) using standard Affymetrix instruments, protocols and software (obtained from Shanghai Bio Corp.) as described in previously (6) with three

biological replicates. The criteria for identifying differentially expressed genes are p-value <0.05 and the absolute value of log2 (fold change)⩾1.

GUS histochemical staining

For the β-glucuronidase (GUS) reporter gene construct, 3,030-bp genomic fragment corresponding to sequence 5′ of the ATG start site of the *BPH9* gene was inserted into the *Sal*I and *Nco*I site of the binary vector pCAMBIA1301 to generate *BPH9* promoter-GUS fusion construct. This P_{BPH9}*::GUS* fusion vector was transformed into Kasalath. GUS staining of sections of T_2 transgenic plant leaf sheaths was done following the standard procedures and observed by light microscopy (7). GUS activity was measured by monitoring the cleavage of the β-glucuronidase substrate 4-methylumbelliferylβ-d-glucuronide (MUG, Sigma). The experiments were repeated three times.

Protoplast transient expression assays

Rice protoplasts were isolated from 10-day-old plants as previous described (8). The Renilla luciferase gene (LUC) expressed under the control of 35S promoter was used as a reporter to monitor protoplast viability. Specific combinations of constructs were co-transfected together with the LUC plasmid into rice protoplasts by polyethylene glycol (PEG) method (9, 10, 11). Luciferase activity was measured 40 h after transfection using a luciferase assay system (Promega), and the reduction in luminescence was compared with a control of *GFP* expression vector-transfected protoplasts. For cell viability assay, the protoplasts were transfected with the indicated plasmids and incubated overnight. For visualizing live cells, protoplasts were co-stained with 220 μg/mL fluorescein diacetate (FDA). Each protoplast sample was scored under fluorescence microscope (FV1000, Olympus) in at least 10 randomly selected microscopic fields. The concentrations of the plasmid and protoplasts used for transformation were kept at the same level for each experiment. The experiment was repeated at least five times with equivalent results.

Protoplasts preparation for subcellular localization experiments were the same as the description above. After transfection, the protoplasts were observed under an Olympus Fluoview FV1000 Laser Scanning Confocal Microscope. Nucleus marker was bZIP63 as described previously (12). EXPO marker was EXO70E2 (13). The Golgi marker has been described previously as Man49-CFP and the ER marker was created by first inserting a synthetic oligonucleotide encoding HDEL (5’-AGATCTCATGACGAGCTGTAACTGCAGTCTAGA) at the C-terminus of the FP genes and subsequently adding the signal peptide of AtWAK2 as described

previously (14). The peroxisomes marker were created by adding the peroxisomal targeting signal 1 (PTS1, Ser-Lys-Leu) at their C-termini of the FP genes as described previously (14). The plastid marker was based on the targeting sequence (first 79 aa) of the small subunit of tobacco rubisco as described previously (14).

Sequencing and phylogenetic analysis

Sequence information of BPH18 from IR65482-7216-1-2 and BPH2/26 from ASD7/ADR52 were obtained from NCBI database. *BPH9* alleles from other 114 rice accessions were sequenced according to *BPH9* alleles in cultivated rice Nipponbare, 9311 and Pokkali or from BGI. *O. barthii* sequence was obtained from Gramene website (http://ensembl.gramene.org/genome_browser/index.html). Sequences were analyzed as previously described (15).

Agronomic performance test

To investigate the effect of *BPH9* on agronomic performance, 9311 and NIL-*BPH9* plants were grown in Wuhan, China, under a standard field management regime for the region. The experiment was conducted in a randomized block design with three replications. Seedlings, 30-day-old, of all experimental materials were transplanted in the field with 16.7-cm spacing between plants within each line and 26.7 cm between rows. At harvest, the middle six plants in the central row of each plot were sampled for trait measurement. The following agronomic traits were measured: plant height, number of panicles per plant, number of filled grains per panicle, number of spikelets per panicle, 1,000-grain weight (in grams) (16).

1. Jing, SL. *et al.*, (2012) Development and use of EST-SSR markers for assessing genetic diversity in the brown planthopper (*Nilaparvata lugens* Stål). *Bull Entomol Res* 102:113-22
2. Huang, Z., He GC., Shu LH., Li X., Zhang Q. (2001) Identification and mapping of two brown planthopper resistance genes in rice. *Theor Appl Genet*102:929–934.
3. Du B, et al. (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. *Proc Natl Acad Sci USA* 106(52): 22163-22168.
4. Johnson, G. A., Mantha, S. V., & Day, T. A. (2000). A spectrofluorometric survey of UV-induced blue-green fluorescence in foliage of 35 species. *J Plant Physiol*, 156(2), 242-252.
5. Aldemita RR, Hodges TK (1996) *Agrobacterium tumefaciens* mediated transformation of *japonica* and *indica* rice varieties. Planta 199: 612–617.
6. Jing, SL. *et al.* (2014) Genome-wide mapping of virulence in brown planthopper

identifies loci that break down host plant resistance. *PLoS One* 9: e98911.
7. Pan, YF. *et al.* (2014) Genes associated with thermo sensitive genic male sterility in rice identified by comparative expression profiling. *BMC Genomics* 15: 1114.
8. Jefferson, R.A., Bevan, M. & Kavanagh T. (1987) The use of the Escherichia coli beta-glucuronidase as a gene fusion marker for studies of gene expression in higher plants. *Biochem Soc Trans* 15:17-8.
9. Zhang, Y., *et al.* (2011) A highly efficient rice green tissue protoplast system for transient gene expression and studying light/chloroplast-related processes. *Plant Methods* 7: 30.
10. Li X, et al. (2005) Flagellin induces innate immunity in nonhost interactions that is suppressed by *Pseudomonas syringae* effectors. *Proc Natl Acad Sci USA* 102(36): 12990-12995.
11. Cesari, S. *et al.* (2014) The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *EMBO J* 33:1941-59.
12. Walter M, Chaban C, Schütze K, et al. (2004) Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. *Plant J* 40(3): 428-438.
13. Lin Y, et al. (2015) Exocyst-Positive Organelles and Autophagosomes Are Distinct Organelles in Plants. *Plant Physiol* 169(3): 1917-1932.
14. Nelson, B. K., Cai, X., & Nebenführ A. (2007) A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. *Plant J* 51:1126-1136
15. Du H, Ouyang, Y., Zhang, C. & Zhang, Q. (2011) Complex evolution of *S5*, a major reproductive barrier regulator, in the cultivated rice *Oryza sativa* and its wild relatives. *New Phytol* 191:275-87.
16. You, A., *et al.* (2006) Identification of QTLs across recombinant inbred lines and test cross populations for traits of agronomic importance in rice. *Genetics* 172: 1287–1300.

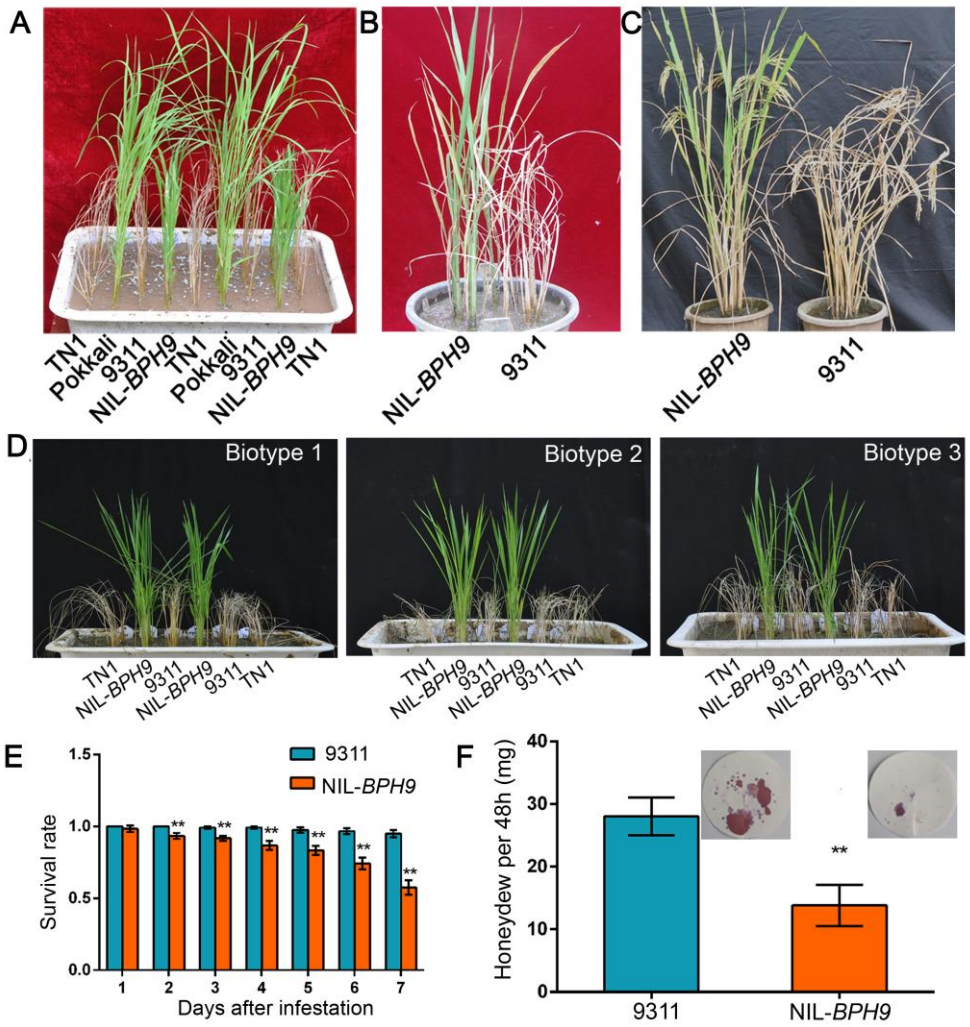

Fig. S1. Characterization of resistance of NIL-*BPH9* to BPH.

(A-C) BPH resistance of NIL-*BPH9* and 9311 at various developmental stages. (A) seedling stage (B) tillering stage (C) mature stage. (D) NIL-*BPH9* shows a high level of resistance to BPH biotypes 1, 2, and 3. NIL-*BPH9*, a near-isogenic line harboring *BPH9* in the 9311 background; 9311 and TN1, susceptible varieties. (E) Survival rates of BPH on NIL-*BPH9* and 9311 plants in a non-choice feeding experiment (**P <0.01 by Student's t test, n=15). (F) Measurement of honeydew excreted from BPH insects feeding on NIL-*BPH9* and 9311 plants for 2 days by weighing and staining methods. (**P <0.01 by Student's t test, n>60).

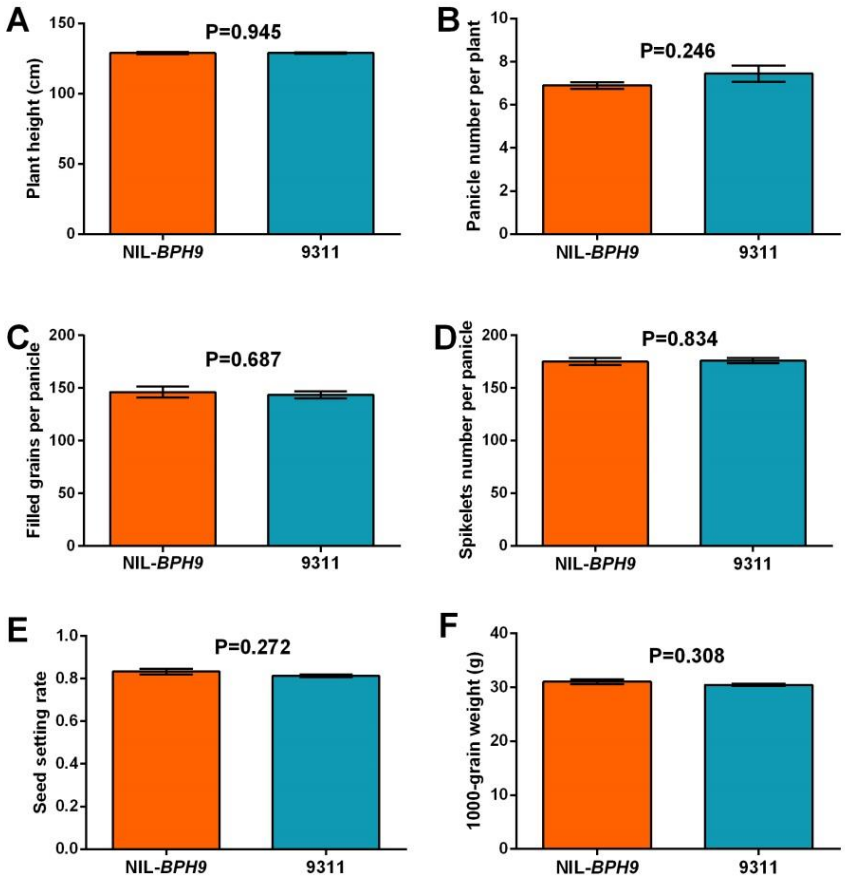

Fig. S2. Characterization of agronomic traits of NIL-*BPH9* and 9311.

(**A**) Plant height (cm); (**B**) Number of panicles per plant; (**C**) Number of filled grains per panicle; (**D**) Number of spikelets per panicle; (**E**) Seed setting rate; (**F**) 1,000-grain weight. All data are means ± SEM (n=6), and the P-values were produced by Student's t test.

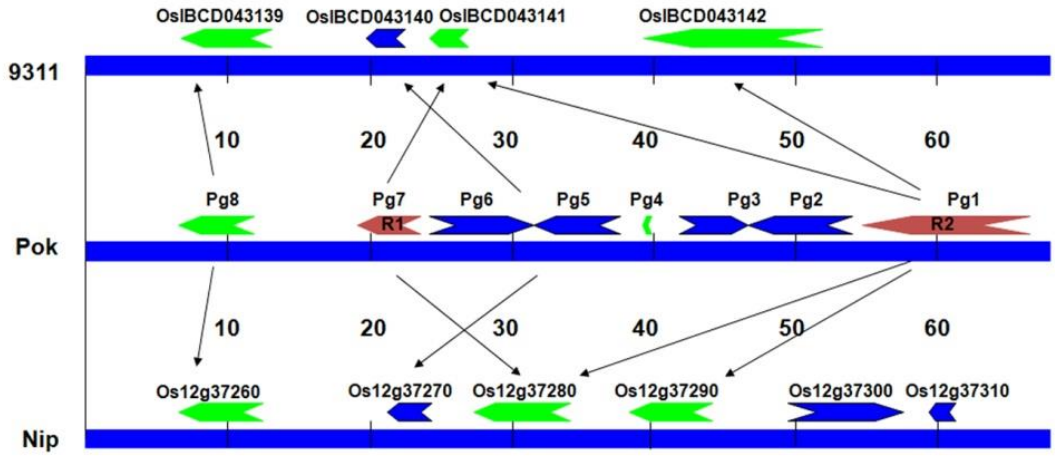

Fig. S3. Comparison of genomic sequences of the *BPH9* regions showing significant sequence diversity among Pokkali (Pok), 9311, and Nipponbare (Nip). In the resistant parental variety Pokkali, *Pg1-Pg8* are eight annotated genes in the 68-kb *BPH9* candidate region predicted by FGENESH. Blue arrows represent predicted retrotransposon sequences. Red arrows represent the two candidate genes of *BPH9*. *R1* is predicted to encode a leucine-rich repeat-containing protein and *R2* is predicted to encode an NB-ARC domain-containing protein. RACE-PCR analysis showed that the two putative genes in 9311 (OsIBCD043141 and OsIBCD043142) and Nip (Os12g37280 and Os12g37290) were actually one gene corresponding to *R2*. Gene annotations of 9311 and Nipponbare were obtained from RiceGE (http://signal.salk.edu/cgi-bin/RiceGE). The numbers indicate nucleotide sequence lengths (in kb).

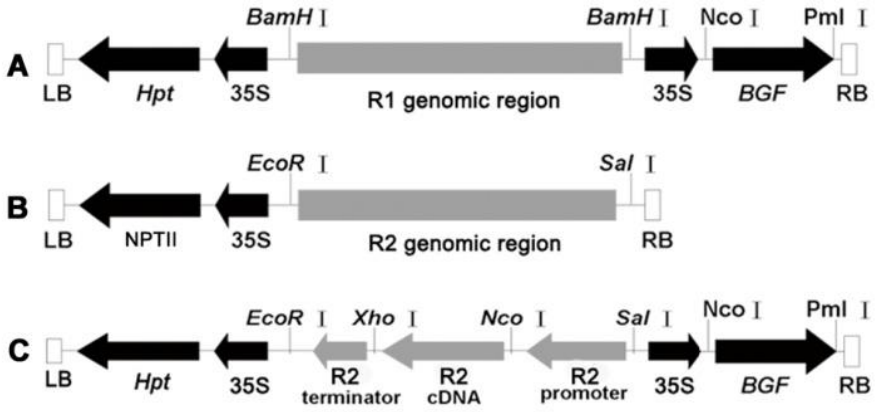

Fig. S4. Schematic representation of complementation vector constructs for *BPH9* candidate genes *R1* and *R2*.

(**A**-**B**) Genomic complementation constructs of *R1* (**A**) and *R2* (**B**). Genomic regions of *R1* (11.3 kb) and *R2* (15.6 kb) containing their native promoters were cloned into modified pCAMBIA1301 and pCAMBIA2300, respectively. Restriction sites used in the process are indicated above. (**C**) The *R2* coding region, fused with its own promoter and terminator (the 3'UTR), was cloned into modified pCAMBIA1301 using three pairs of restriction sites (*Eco*RI-*Xho*I, *Xho*I-*Nco*I, and *Nco*I-*Sal*I).

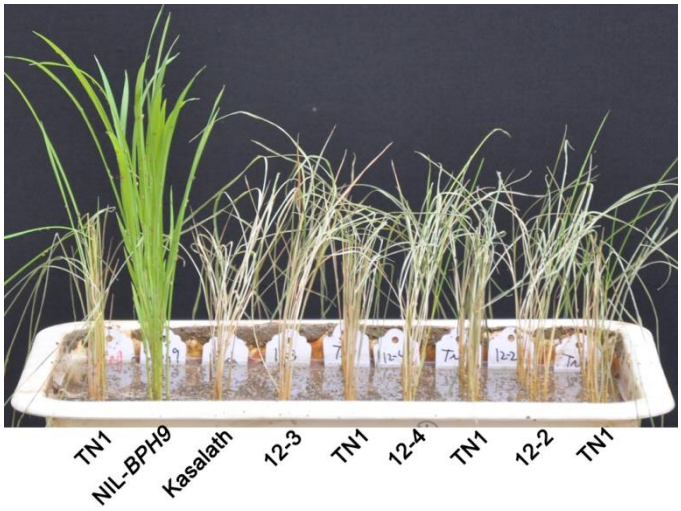

Fig. S5. Complementation tests of the candidate gene *R1*.

Rice plants were infested with BPH and examined after seven days. The *R1* transgenic and the susceptible variety Kasalath plants all died, while NIL-*BPH9* survived, showing that *R1* transgenic lines were not resistant. Kasalath, susceptible variety; 12-2, 12-3 and 12-4, independent *R1* transgenic lines. TN1 and NIL-*BPH9* were used as the susceptible and resistant control, respectively.

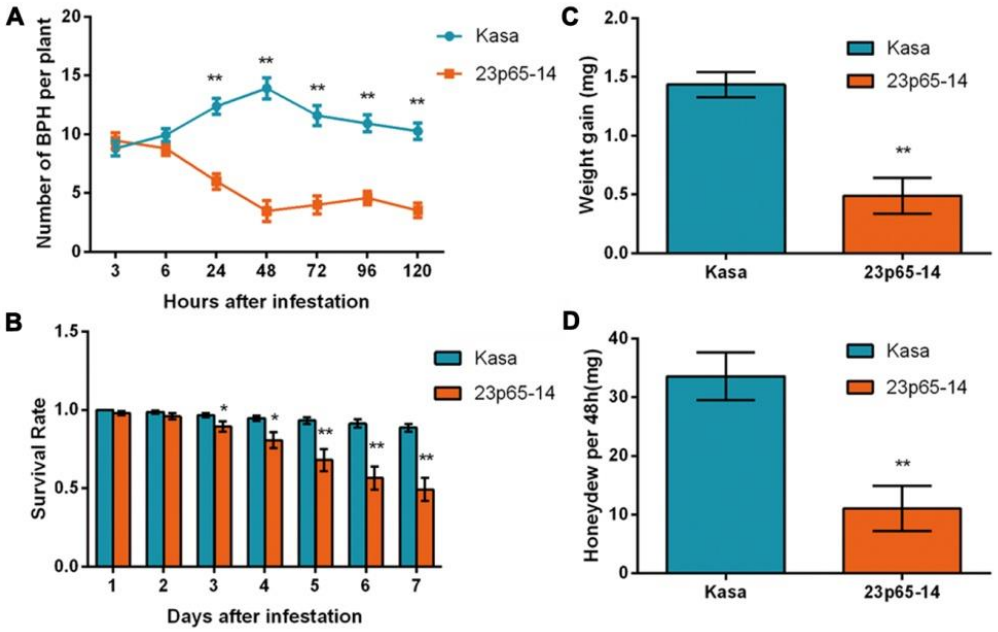

Fig. S6. Antixenosis and antibiosis effects of *BPH9*-transgenic plants on BPH insects.

(**A**) Settling of BPH insects on *BPH9*-transgenic plants and on Kasalath in a two-host choice test. (**B**) BPH survival rate on *BPH9*-transgenic plants and on Kasalath in a non-choice feeding experiment. (**C**) Weight gain of BPH insects after feeding on *BPH9*-transgenic plants and on Kasalath for 2 days. (**D**) Measurement of honeydew excreted from BPH insects on *BPH9*-transgenic plants and on Kasalath. Kasalath, susceptible background variety; 23p65-14, transgenic lines harboring the full genomic region of *BPH9*. All data are means ± SEM (*P<0.05 and **P <0.01 by Student's t test, **A**, **B**, n=15; **C**, **D,** n=34).

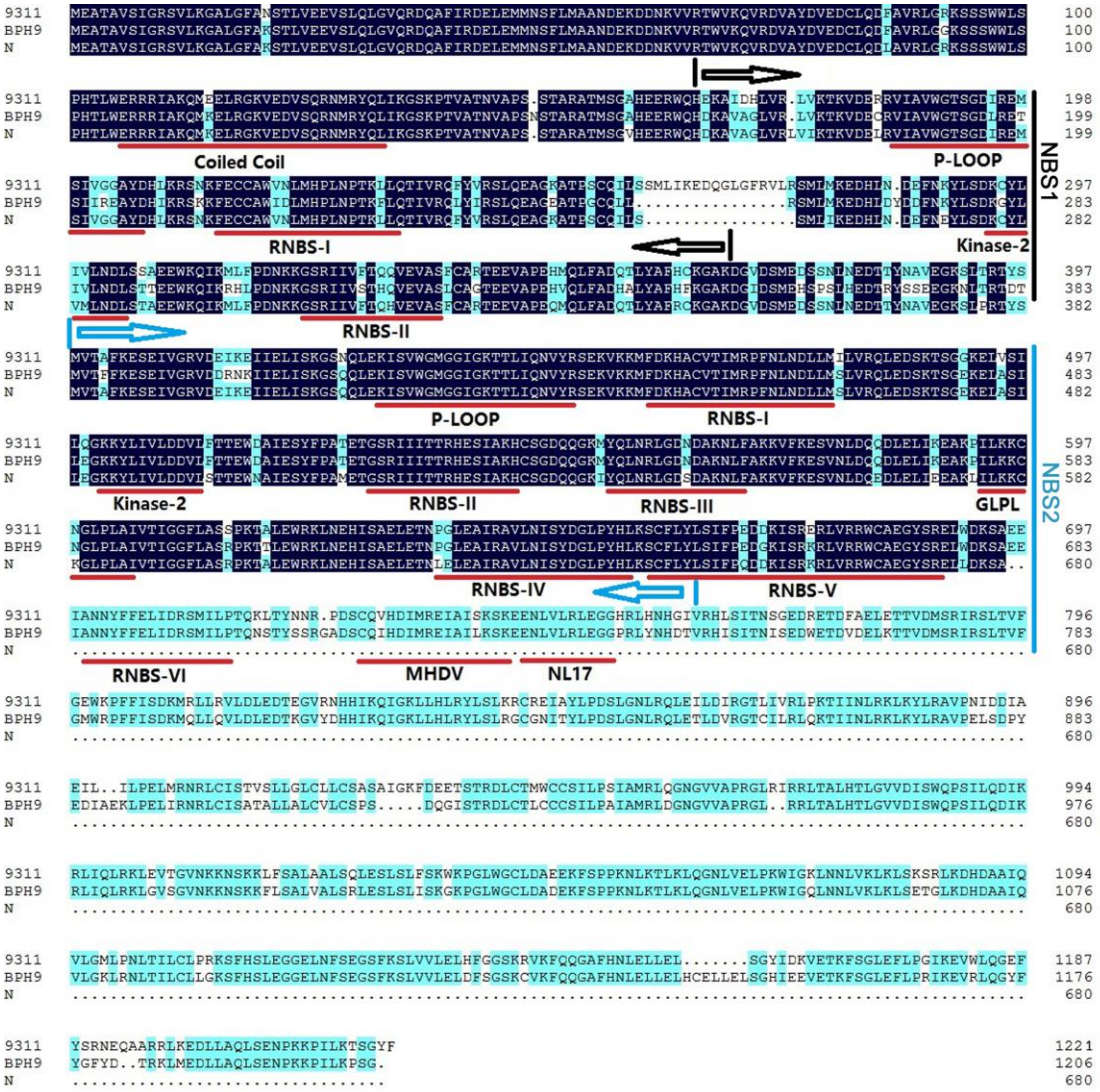

Fig. S7. Amino acid sequence comparison of BPH9 and susceptible alleles in 9311 and Nipponbare.

The NBS1 domain is illustrated between two black arrows above the amino acid sequences. The NBS2 domain is illustrated between two blue arrows above the amino acid sequences. The conserved sequence motifs are underlined with their motif names indicated.

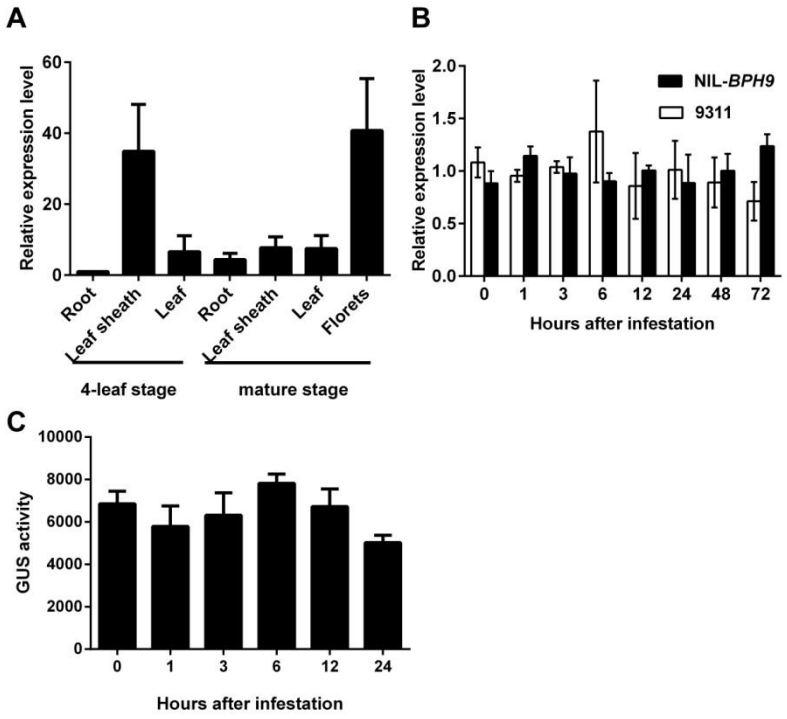

Fig. S8. Expression analysis of *BPH9*.

(**A**) qRT-PCR analysis of *BPH9* tissue-specific expression. Various tissues from rice plants at the four-leaf stage and mature stage were analyzed. (**B**) qRT-PCR analysis of *BPH9* expression in rice leaf sheaths at the indicated time points after BPH infestation. (**C**) Measurement of GUS activity in P_{BPH9}::GUS reporter transgenic lines at the indicated time points after BPH infestation. All data are means ± SEM based on three biological replicates and three technical replicates.

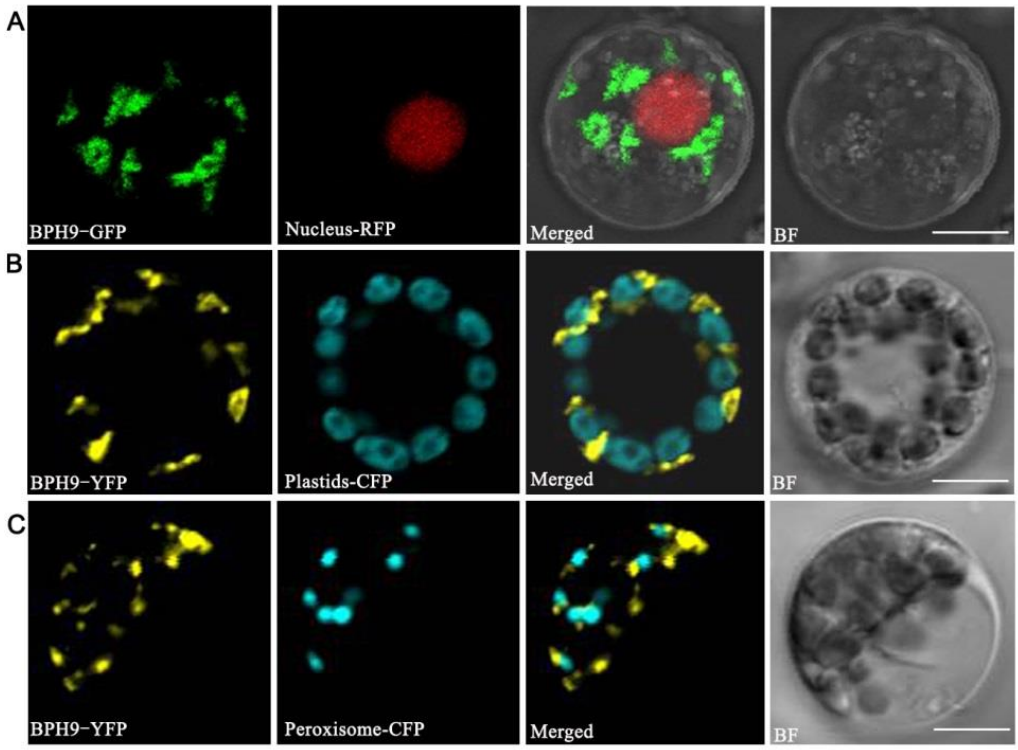

Fig. S9. Transient co-transformation of BPH9 and organelle markers in rice protoplast.

BPH9 was co-transformed with nuclear marker (bZIP63) (**A**), plastids marker (rubisco79) (**B**), and peroxisomes marker (FP-PTS1) (**C**) in rice protoplast. Non-overlapping fluorescence signals exclude the possibility that BPH9 localize to these organelles. BF, bright field; scale bar = 5 μm.

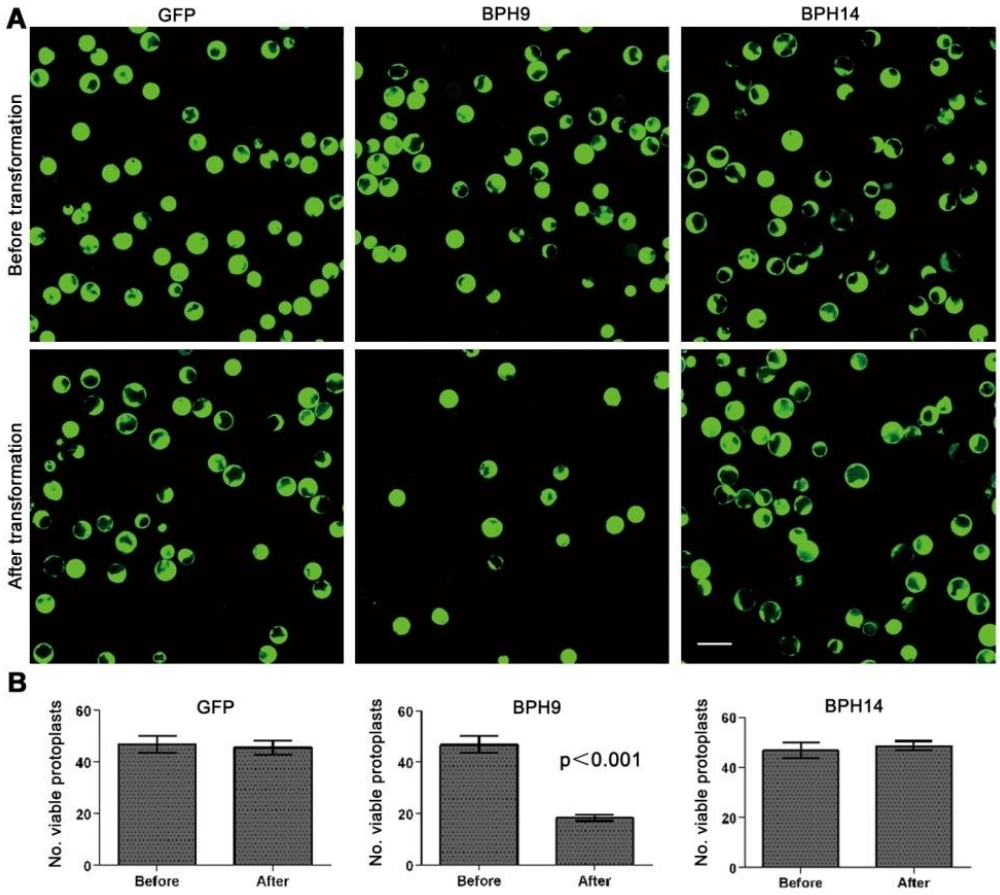

Fig. S10. *BPH9* induced cell death in rice protoplasts.

(**A**) Representative images of fluorescein diacetate (FDA)-stained viable rice protoplasts before and after transformation with *GFP*, *BPH9*, and *BPH14*, respectively. Images were taken 20 h after transformation. Bar=20 μm. (**B**) Number (No.) of viable rice protoplasts before and after transformation with *GFP*, *BPH9*, or *BPH14* was scored after FDA staining. Average values and standard errors were calculated from at least five independent experiments and at least 10 randomly selected microscopy fields were scored per experiment. P-values were determined by Student's t test.

A

Comparison	Number of probe&gene sets differentially expressed (P<0.05, logFC≥1)					
	Total numbers		logFC≥1			
			Up		Down	
	probe	gene	probe	gene	probe	gene
B3/B0	2535	1230	1310	643	1225	587
B6/B0	3029	1523	1292	682	1737	841
93/90	2254	1645	874	626	1380	1019
96/90	2186	1516	840	584	1346	932
B0/90	2348	1159	1139	587	1209	572
B3/93	2484	1207	1492	742	992	465
B6/96	2626	1202	1476	708	1150	494

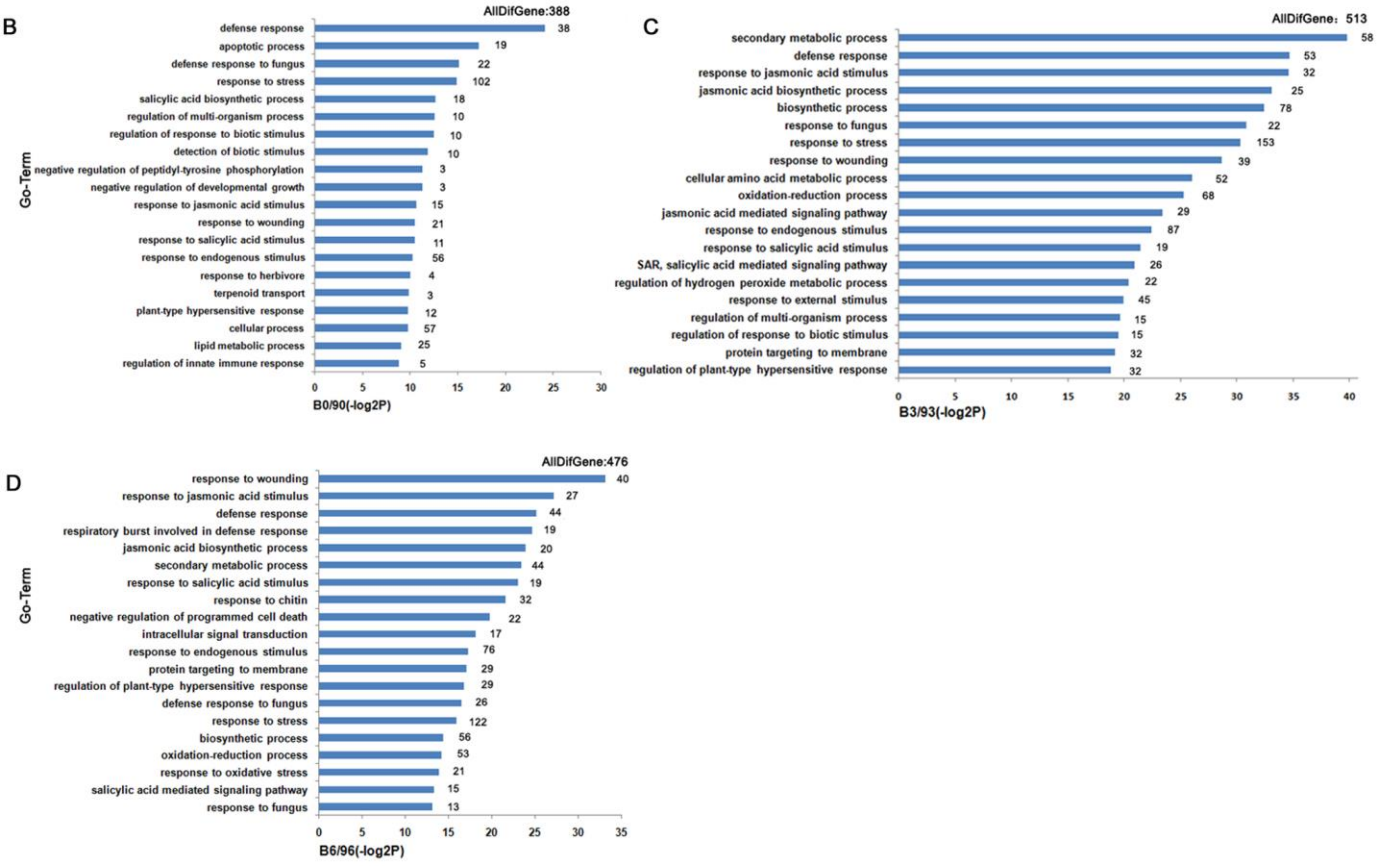

Fig. S11. GO analysis of differentially expressed genes between NIL-*BPH9* and 9311 at P < 0.001.

(**A**) List of differential expression probes and genes between rice cultivars NIL-*BPH9* and 9311 in response to BPH attack revealed by the microarray analysis. (B-D) Gene ontology (GO) terms that were up-regulated in the NIL-*BPH9* plants in comparison with 9311 at 0 (B), 3 (**C**) and 6 hours (**D**) after BPH infestation. The number of differentially expressed genes in the corresponding GO terms are tagged behind the GO-Term. GO terms were ranked by their P-value of significance (-log2P). The longer the bar (GO term), the more significantly is the gene up-regulated at the indicated time point.

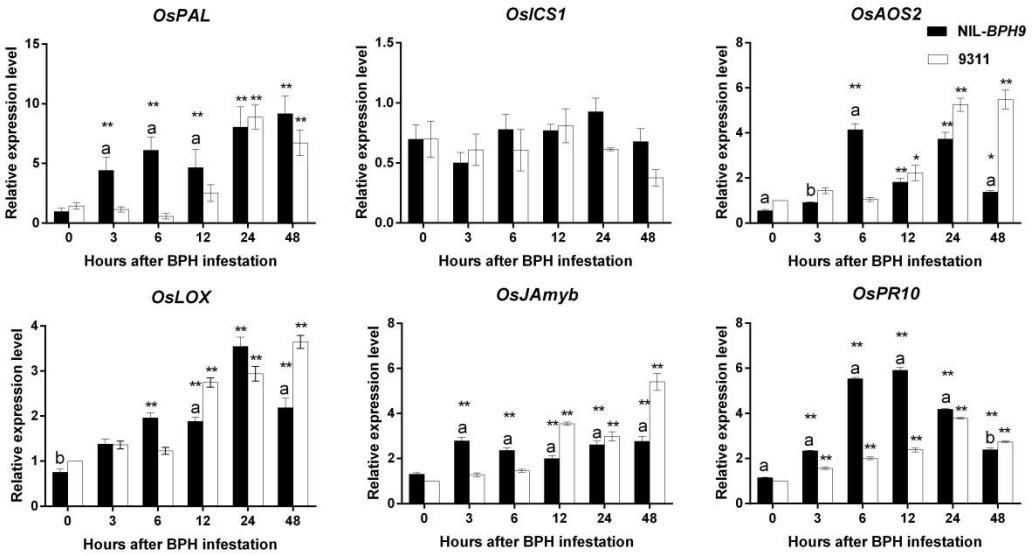

Fig. S12. Expression analysis of phytohormone-response genes and defense-related genes. *OsPAL* and *OsICS1* are involved in SA-signaling; *OsLOX*, *OsAOS2* and *OsJAmyb* in JA-signaling; *OsPR10* (also known as *OsPBZ1*) is related to defense that is associated with both SA- and JA-signaling pathways. Gene expression levels were normalized using two internal reference genes: *OsTBP* and *OsSDHA*. Bars represent means ± SEM of three biological replicates, each containing a pool of 15 plants. The 'a' and 'b' indicate that a significant difference was detected between 9311 and NIL-*BPH9* at the same time point after BPH infestation ($P < 0.01$ or $P < 0.05$, respectively). Asterisks indicate that a significant difference was detected between none-infested plants (0h) and attacked plants at the indicated time points (**$P < 0.01$ and *$P < 0.05$).

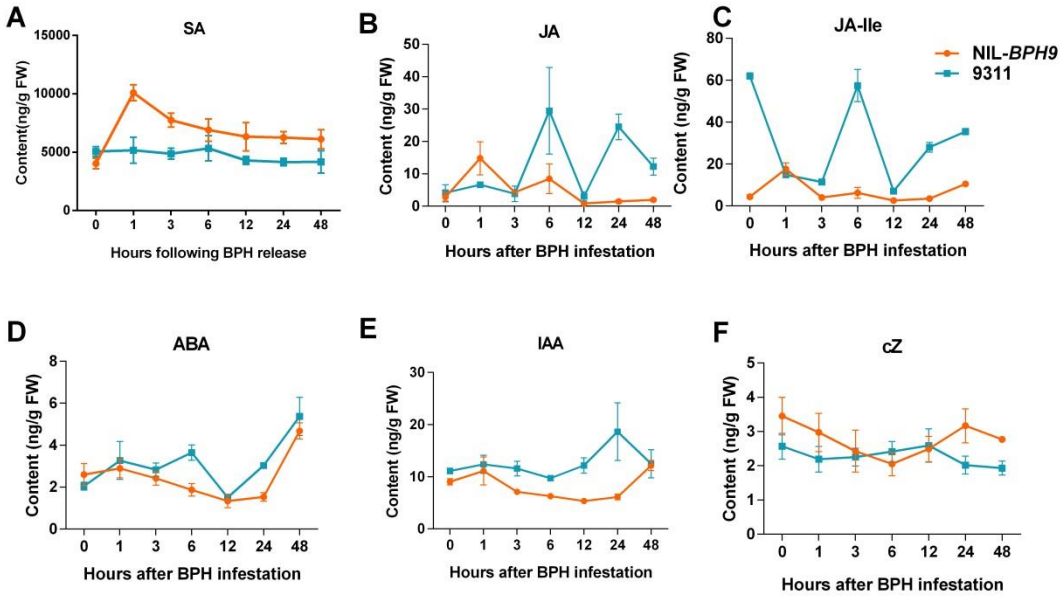

Fig. S13. Measurements of plant hormones.

Quantities of indicated hormones detected in 9311 and NIL-*BPH9* plants (ng/g.FW) after indicated hours (0-72) of infestation by BPH. (**A**) SA; (**B**) JA; (**C**) JA-Ile; (**D**) Abscisic acid; (**E**) Auxin; (**F**) cis-zeatin. Data are means ± SEM, n=3.

A

	BPH26	BPH2	BPH9	BPH18	BPH21	BPH1	BPH10	BPH7
BPH7	94.85	94.85	94.75	97.83	97.83	97.83	97.83	100.00
BPH10	94.46	94.46	96.05	100.00	100.00	100.00	100.00	
BPH1	94.46	94.46	96.05	100.00	100.00	100.00		
BPH21	94.46	94.46	96.05	100.00	100.00			
BPH18	94.46	94.46	96.05	100.00				
BPH9	91.32	91.32	100.00					
BPH2	100.00	100.00						
BPH26	100.00							

B

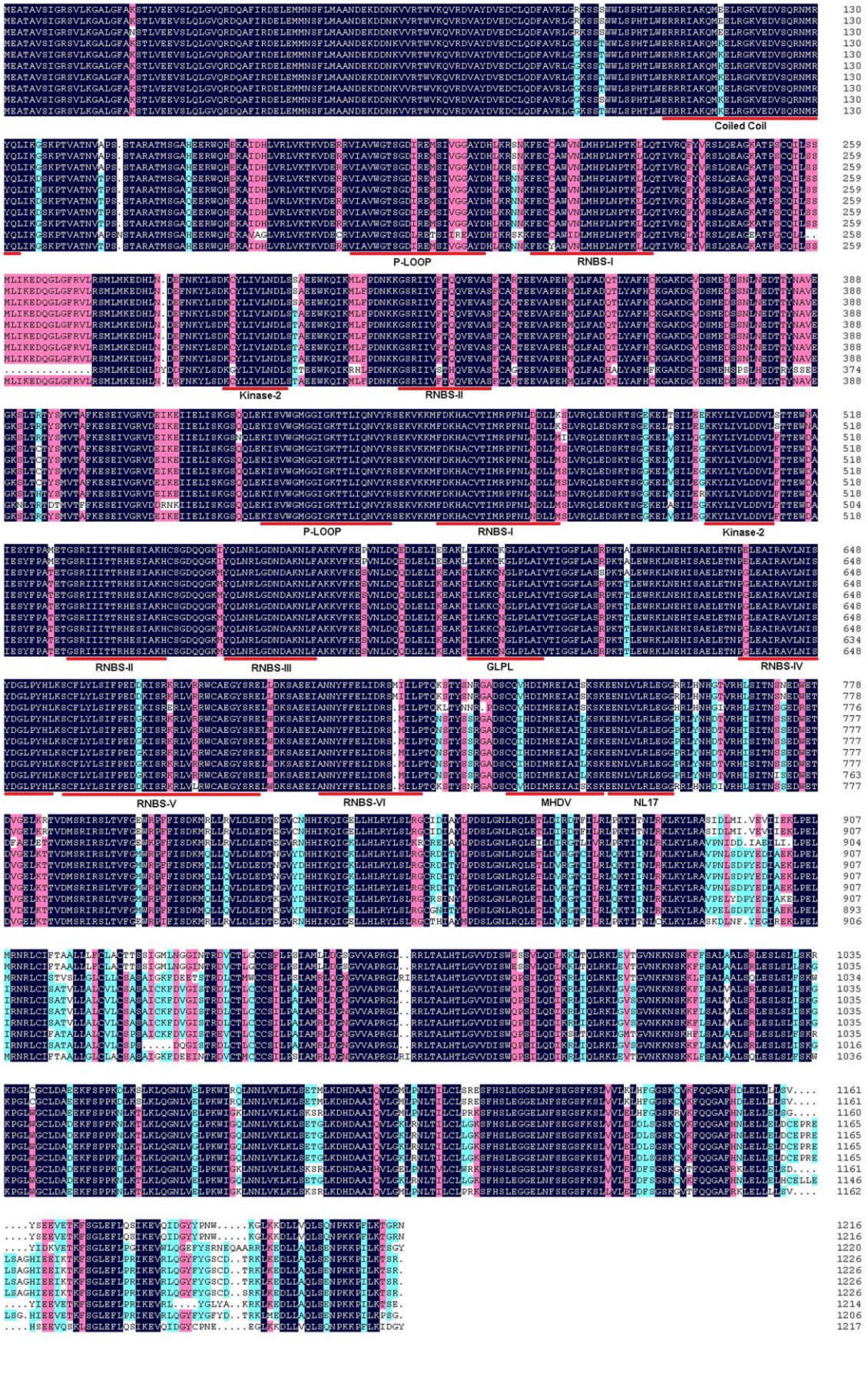

Fig. S14. Sequence comparison of resistant and susceptible alleles of *BPH1/9*.

(**A**) Percent identity of nucleotide sequence of the eight BPH-resistance genes clustered on chromosome 12L. (**B**) Alignment of amino acid sequences of the BPH-resistance genes clustered on chromosome 12L. 9311 carries the susceptible allele. Dark blue indicates 100% identity of amino acid sequences. Pink indicates identity of 75-100% and light blue indicates identity of less than 75% to 50%. The conserved sequence motifs are underlined with their motif names indicated. *Indica* rice varieties Babawee and Rathu Heenati that do not possess the *BPH1/9* resistance gene were obtained from Yasumori Tamura et al. (2014).

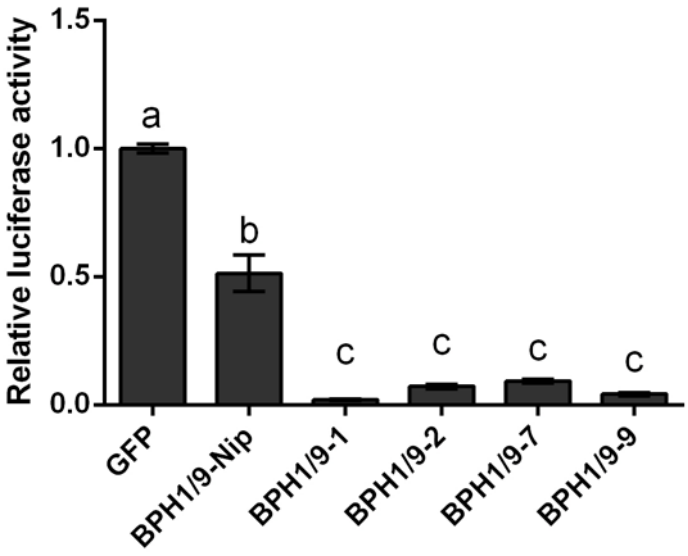

Fig. S15. Luciferase (LUC) activity in rice protoplasts co-expressing LUC and *BPH1/9* alleles.

Different characters above the bars indicate a significant difference by one-way analysis of variance and LSD test as post hoc analysis. All data are means ± SEM of three technical replicates. The experiment was repeated five times with equivalent results.

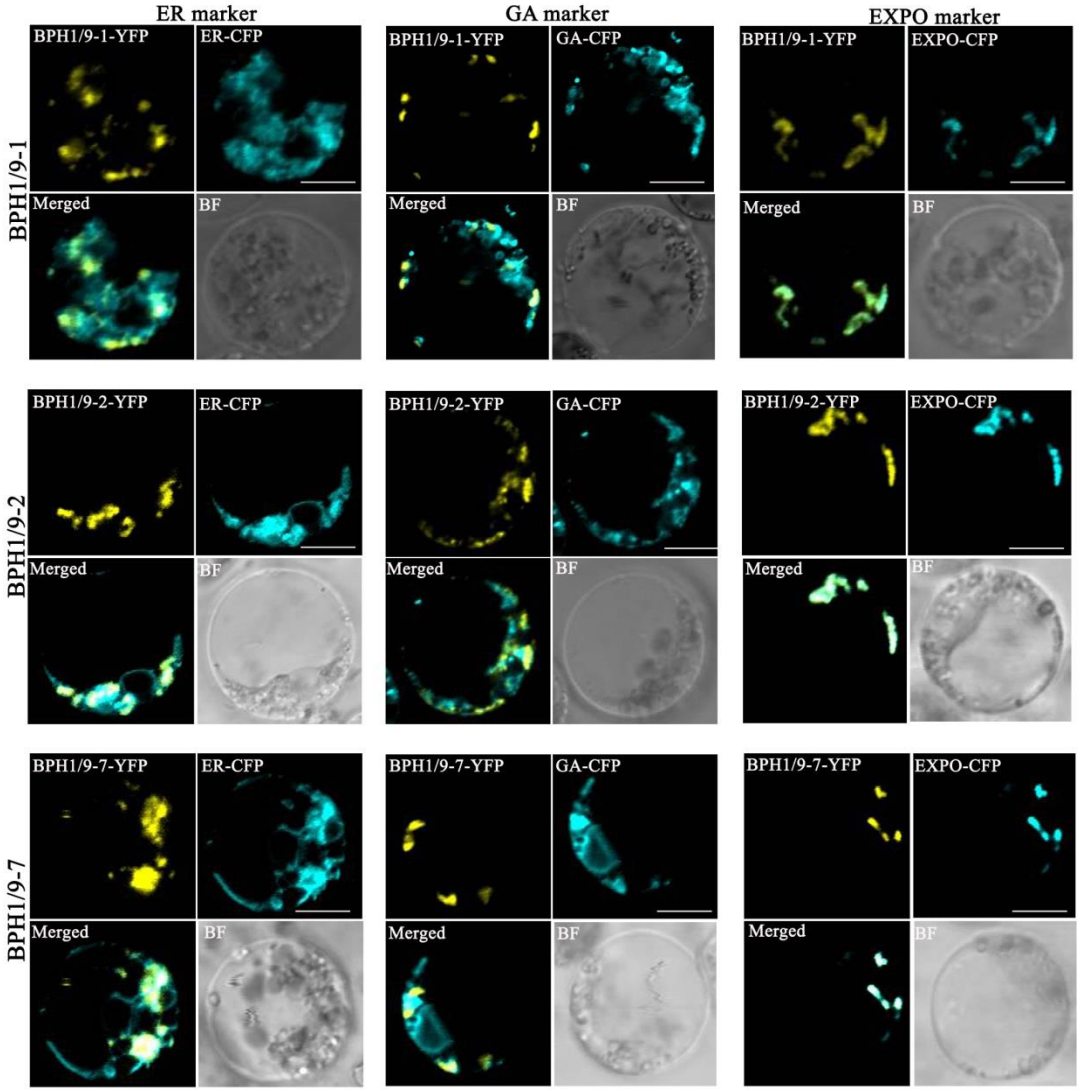

Fig. S16. Subcellular localization of the proteins encoded by the *BPH1/9-1*, *BPH1/9-2*, and *BPH1/9-7* in rice protoplasts.

BPH1/9-1, BPH1/9-2, BPH1/9-7 share the same subcellular localization as BPH1/9-9. YFP tagged BPH1/9-1, BPH1/9-2, and BPH1/9-7 proteins were co-transformed with the endoplasmic reticulum (ER) marker, Golgi apparatus (GA) marker and exocyst-positive organelle (EXPO) marker. BF, bright field. Scale bars, 5 μm.

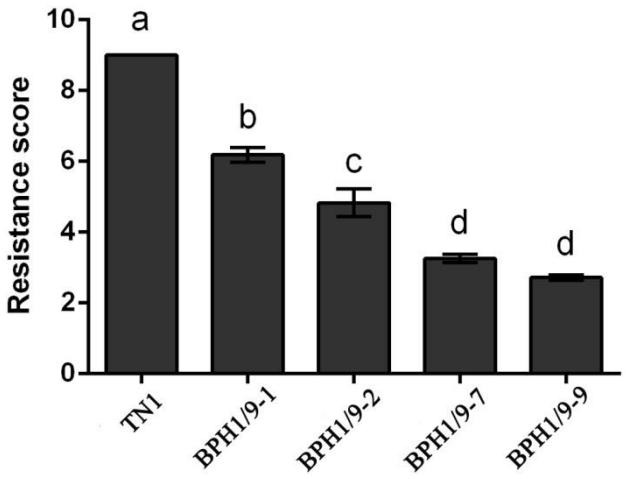

Fig. S17. The four types of BPH alleles that confer diverse levels of resistance to wild populations of BPH.

Wild populations of BPH were collected in the rice field in Wuhan, China. The resistance scores of rice plants were evaluated in seedling bulk tests with lower scores indicating higher resistance. TN1 is a susceptible control. Different characters above the bars indicate a significant difference by one-way analysis of variance and LSD test as post hoc analysis. All data are means ± SEM of 20 biological replicates. The experiment was repeated twice with equivalent results.

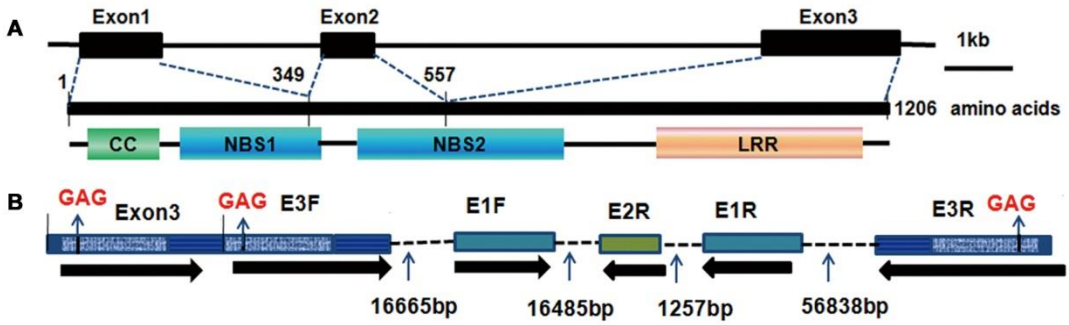

Fig. S18. Coding region of *BPH9* and its homologous fragments in the wild rice species *O. barthii.*

(**A**) Structure of *BPH9* and the corresponding encoded polypeptide. (**B**) Homologous fragments of *BPH9* in the wild rice *O. barthii*. Two homologs of exon1 were found in O. *barthii* (E1F and E1R). Light blue boxes indicate the two exon1-homologous fragments; the forward one is referred to as E1F, the reverse one as E1R. The green box indicates exon2- homologous fragment (E2R) inserted in reverse. Exon3-homologous fragments (E3F and E3R) are shown in the same way as in Fig. 4D. The forward homologous fragment is referred to as E3F, and the reverse one as E3R. Black arrows indicate insertion into genomic DNA in the forward or reverse orientation. Sequences exhibiting more than 90% sequence identity are shown in identical colors. The site 2041T forms a stop codon TAG in *japonica* Nipponbare while all the homologous fragments in *O. barthii* are GAG.

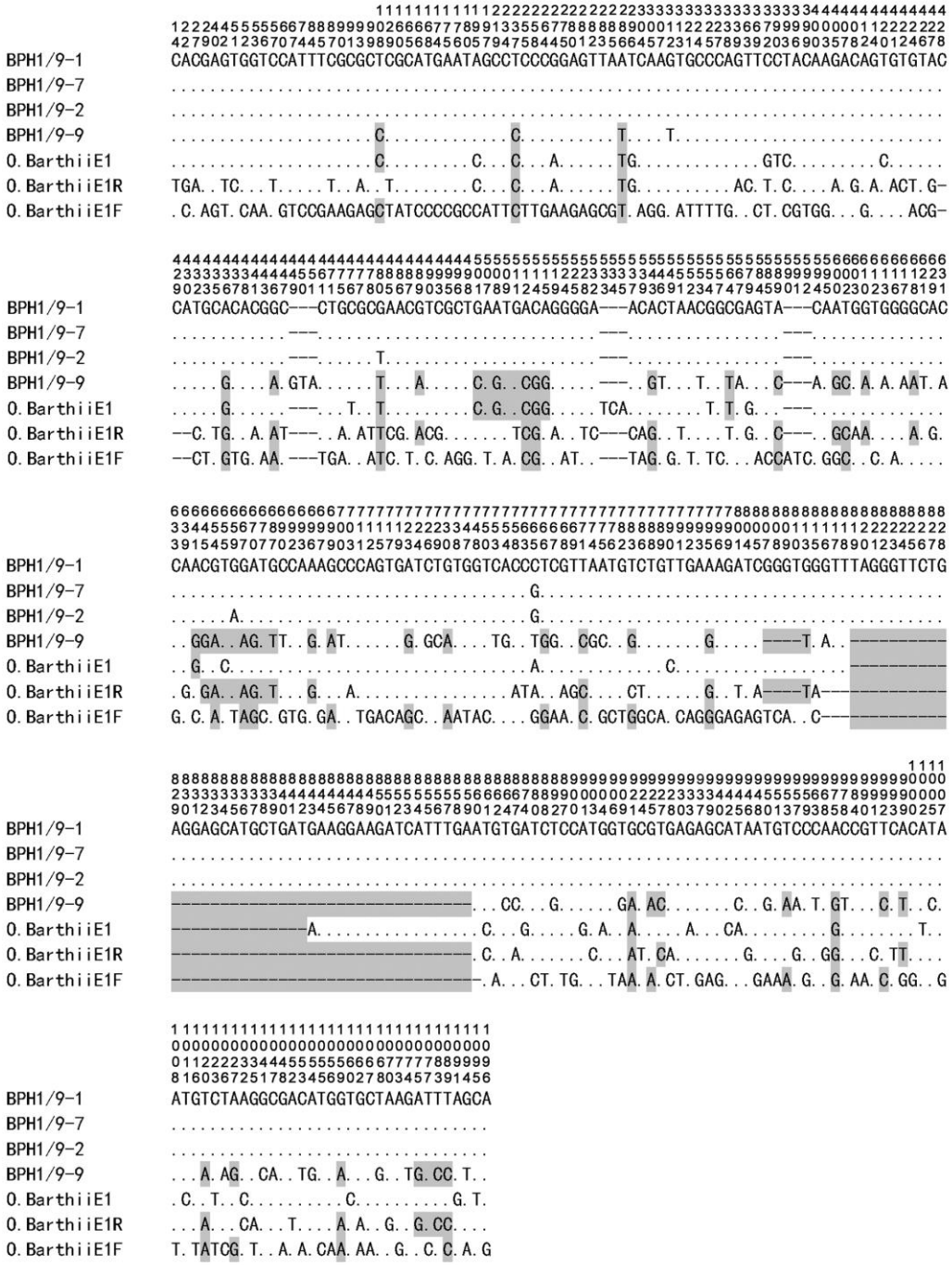

Fig. S19. The patchwork distribution of nucleotide polymorphisms in exon1 of *BPH9* alleles and homologous fragments in the wild rice species *O. barthii.*

Only polymorphic sites in the exon1 region are shown. The numbers presented vertically at the top indicate position number of exon1 variable nucleotides. Two homologs of exon1 were found in *O. barthii*, one in forward and the other in reverse orientations. The forward homologous fragment of exon1 in *O. barthii* is referred to as E1F, and the reverse one as E1R. Shaded letters indicate identical residues in

different fragments. Dots represent nucleotides that conform to those in the reference sequence of *BPH1/9-1*. Gaps in the alignment are represented by dashes.

Table S1 Molecular marker genotypes and phenotypes of the selected recombinants used for fine mapping.

Parents and recombinants	RM28438	InD2	InD14	PvuI	RsaI	RM28466	RM28481	RM28486	Phenotype	Resistance scores
9311	S	S	S	S	S	S	S	S	S	8.8
3A1	S	S	S	S	S	S	H	H	S	8.7
3A2	H	H	H	H	H	S	S	S	H	4.3
3A3	H	H	H	H	R	R	R	R	H	5.3
3A4	R	R	R	R	H	H	H	H	R	2.9
3A5	R	R	R	R	R	H	H	H	R	3.3
3A6	S	S	H	H	H	H	H	H	H	5.3
3A7	H	H	S	S	S	S	S	S	S	8.7
3A8	R	R	H	H	H	H	H	H	H	4.8
3A9	H	S	S	S	S	S	S	S	S	8.6
Pokkali	R	R	R	R	R	R	R	R	R	1.9

R, homozygous for the allele from Pokkali; S, homozygous for the allele from 9311; H, heterozygous for alleles from the two parents. Pokkali and 9311 are the resistant parent and the susceptible parent, respectively.

Table S2 Rice varieties used for sequencing and phylogenetic analysis

No.	Accession No.	Accession name	Origin	Subspecies	Haplotype
1	HBY1040	Nanoay P.A	Argentina	*japonica*	H1
2	IRGC 8555	DZ78	Bangladesh	*indica*	H1
3	IRGC 60542	HAISHA CAMAN	Bangladesh	_	H1
4	IRGC 32399	Phudugey *4	Bhutan	*japonica*	H1
5	IRGC 50448	Canella De Ferro	Brazil	*japonica*	H1
6	IRGC 38994	Bico Branco	Brazil	*japonica*	H1
7	IRGC 51250	Ai-Chiao-Hong	China	*indica*	H1
8	IRGC 1107	Ta Hung Ku	China	*japonica*	H1
9	WD-17772	JW 60	India	*indica*	H1
10	IRGC 45975	Kalamkati	India	*indica*	H1
11	IRGC 6307	Jhona 349	India	*indica*	H1
12	IRGC 9060	JC101	India	*japonica*	H1
13	HBY1024	Djanda Mandja	Indonesia	*japonica*	H1
14	IRGC 17757	Jambu	Indonesia	*japonica*	H1
15	HBY1025	Padi Ladang Ase Polo Komek	Indonesia	*indica*	H1
16	IRGC 43675	Trembese	Indonesia	*japonica*	H1
17	IRGC 43325	Arias	Indonesia	*japonica*	H1
18	IRGC 12883	Mehr	Iran	*japonica*	H1
19	RA4952	Firooz	Iran	*japonica*	H1
20	HBY1019	Linguo	Italy	*japonica*	H1
21	HBY1058	IRAT 36	Ivory Coast	*japonica*	H1
22	HBY1240	IRAT 669	Ivory Coast	*japonica*	H1
23	IRGC 2540	Haginomae Mochi	Japan	*japonica*	H1
24	IRGC 418	Norin 20	Japan	*japonica*	H1
25	IRGC 8191	Mansaku	Japan	*japonica*	H1
26	NP	Nipponbare	Japan	*japonica*	H1
27	HBY1241	SLK 2-18-2	Laos	*indica*	H1
28	IRGC 11010	Maintmolotsy	Madagascar	*japonica*	H1
29	IRGC 12793	Kitrana 508	Madagascar	*japonica*	H1
30	WD-11752	Jingnong 1	Malaysia	*indica*	H1
31	WD-14938	Fuzi 102	Nepal	*japonica*	H1
32	HBY1032	Kalijira 245	Pakistan	*indica*	H1
33	IRGC 8244	Davao	Philippines	*japonica*	H1
34	IRGC 26872	Binulawan *11	Philippines	*indica*	H1
35	IRGC 328_	AZUCENA	Philippines	*japonica*	H1
36	HBY1013	Hongse 90	Russia	*japonica*	H1
37	HBY1192	Taichung 65	Taiwan,China	*japonica*	H1
38	IRGC 66756	Lemont	USA	*japonica*	H1
39	HBY1067	R42	USA	*indica*	H1
40	_	Junam	Korea	*japonica*	H1
41	IRGC 31856	KUI SALI	Bangladesh	*japonica*	H2
42	HBY1020	Meiguohuangkedao	USA	*indica*	H3
43	IRGC 51300	Guan-Yin-Tsan	China	*indica*	H4

44	IRGC 25901	Miriti *4	Bangladesh	*indica*	H5
45	IRGC 8231	Gie 57	Vietnam	*indica*	H6
46	IRGC 27762	Leung Pratew *11	Thailand	*indica*	H7
47	WD-11689	Pulumgaman	Myanmar	*japonica*	H8
48	HBY1030	Dumai	India	*indica*	H9
49	WD-11470	Loc dau	Vietnam	*indica*	H10
50	HBY1043	71011	Australia	*indica*	H11
51	HBY1078	Huangsiguizhan	China	*indica*	H11
52	HBY1037	P1790-5-1M-4-5M-1B-3M-B	Colombia	*indica*	H11
53	HBY1257	C.MEDIO 7	Cuba	*indica*	H11
54	HBY1204	ECIA 179-S13	Cuba	*indica*	H11
55	HBY1226	_	India	*indica*	H11
56	HBY1029	B96-Tk-23-5-5-2-2-2	India	*indica*	H11
57	WD-15413	Pusa 33	India	*indica*	H11
58	HBY1225	CISOKAN	Indonesia	*indica*	H11
59	HBY1282	Brenda A-75	Mexico	*indica*	H11
60	WD-11698	Miandianhangu	Myanmar	*indica*	H11
61	WD-13670	X69-56-12-10-6-3	Myanmar	*indica*	H11
62	WD-14947	KK 15-37-C	Nepal	*indica*	H11
63	WD-17932	BG 850-1	Sri Lanka	*indica*	H11
64	HBY1023	Xianluosichi	Thailand	*indica*	H11
65	WD-11711	Daxianluo	Thailand	*indica*	H11
66	HBY1042	K 24	Uganda	*indica*	H11
67	HBY1279	IV di tam lun	Vietnam	*indica*	H11
68	WD-11471	Loc Ruong	Vietnam	*indica*	H12
69	_	ADR52	IRRI	*indica*	H13 (BPH1/9-2)
70	_	ASD7	India	*indica*	H13
71	HBY1055	BR 2029-2-2-2	Bangladesh	*indica*	H14
72	WD-15785	BRC 24-200-5-2-1	Bangladesh	*indica*	H14
73	WD-11995	CR 94-13	India	*indica*	H14
74	WD-15443	RUR 1429	India	*indica*	H14
75	HBY1244	BW 293-2	Sri Lanka	*indica*	H14
76	WD-13641	DM36	Vietnam	*indica*	H14
77	WD-17044	OM 296	Vietnam	*indica*	H14
78	WD-17464	NR601-1-1-6-2	Nepal	*indica*	H15
79	WD-17607	ASDT	India	*indica*	H16
80	HBY1248	2037(Rajahamsal)	India	*indica*	H16
81	HBY1249	Seln 244A6-20	Australia	*indica*	H17
82	HBY1230	JC 78	India	*indica*	H17
83	HBY1243	RNR 67580	India	*indica*	H17
84	HBY1245	Ngatsin	India	*indica*	H17
85	HBY1272	Jaibattey	India	*indica*	H17
86	WD-15185	HPU 741	India	*indica*	H17
87	WD-15445	RNR 52147	India	*indica*	H17
88	WD-15515	UPR 80-1-1-2-1	India	*indica*	H17
89	HBY1273	C 894-21	Philippines	*indica*	H17

90	WD-14405	Burik	Philippines	*indica*	H17
91	HBY1277	Ikong	Vietnam	*indica*	H17
92	WD-11612	No 47	Vietnam	*indica*	H17
93	9311	9311	China	*indica*	H17
94	HBY1159	Aizaizhan	China	*indica*	H18
95	WD-17628	CR 157-392-284	India	*indica*	H19 (BPH1/9-9)
96	IRGC 128020	CR157-392-4	India	*indica*	H19
97	WD-14400	BM 13	Philippines	*indica*	H19
98	BPH9	Pokkalli	Sri Lanka	*indica*	H19
99	HBY1045	Pelde	Australia	*indica*	H20 (BPH1/9-1)
100	HBY1252	Jineiyadao	Guinea	*indica*	H20
101	WD-15206	Inall 658	India	*indica*	H20
102	WD-15448	RNR 74802	India	*indica*	H20
103	WD-15491	TNAU 6464	India	*indica*	H20
104	WD-17636	CSR 13	India	*indica*	H20
105	WD-11962	ARC 7320	India	*indica*	H20
106	HBY1027	IR 10179-23-1-3	IRRI	*indica*	H20
107	WD-16534	Sanjiang	Japan	*indica*	H20
108	HBY1028	NR 10045-20-3-2	Nepal	*indica*	H20
109	WD-13513	Milyamg 30E	Korea	*indica*	H20
110	WD-11848	IR28	Philippines	*indica*	H20
111	WD-17950	KALU KURUWEE	Sri Lanka	*indica*	H20
112	WD-13731	SRRLR76102-26-1-1	Thailand	*indica*	H20
113	_	Mudgo	India	*indica*	H20
114	_	IR65482-4-136	IRRI	*indica*	H20
115	_	IR71033-131	IRRI	*indica*	H20
116	_	IR65482-7216-1-2	IRRI	*indica*	H20
117	_	T12	IRRI	*indica*	H21 (BPH1/9-7)

Table S3 Nucleotide polymorphisms in *BPH9* alleles.

Group	component	Location (nt)	L(bp)	S	π	θ	Tajima'D	Fu&Li'D	Fu&Li'F
All	Total	1-3708	3553	520	0.04421	0.03054	2.04663	1.93743**	2.38028**
	CC	1-495	492	23	0.01537	0.00876	2.18392*	0.98293	1.73215*
	NBS1	496-1233	682	116	0.02982	0.03271	-0.21028	2.41220**	1.52024
	NBS2	1234-2343	1104	86	0.02544	0.01494	2.39305**	1.83363**	2.48989**
	LRR	2344-3708	1275	295	0.07928	0.05131	2.75500**	1.60080*	2.54142**
Indica group	All	1-3708	3553	517	0.04705	0.03226	2.15046*	1.88538**	2.39936**
Japonica group	All	1-3654	3603	45	0.00081	0.00313	-2.73686 **	-5.21245**	-5.19105 **

S: number of polymorphic or segregating sites; π: nucleotide diversity, the average number of nucleotide differences per site between two sequences; θ: Watterson's nucleotide diversity estimator; Tajima's D, Fu and Li's D, and Fu and Li's F statistics were based on the differences between the number of segregating sites and the average number of nucleotide differences (*P＜0.05; ** P＜0.02).

Table S4 List of primers used in this study.

	Locus ID	Forward primer	Reverse primer	Purpose
High-resolution mapping				
InD14		CCACTCTGAAAATCCCAAGC	ACCAGTTAAGTCACGCTCAAA	Map-based cloning
InD2		AACAGACACGTTGCGTCTTG	CTTGCCGCTTAGAGGAGATG	Map-based cloning
RsaI		TCTCGGACGAGCTGCTAAG	CGAGTGGAATTTGTGGAGGT	Map-based cloning
PvuI		GACATGGGGTGTTTTTGGAC	ATGTTTGCAAAACACGGTGA	Map-based cloning
Quantitative RT-PCR analysis				
BPH9-RT		GCCGCCAATGATGAGAAAGA	GCTGAGCCACCAGGATGAAC	RT-PCR
OsTBP	LOC_Os03g45410	AGGTCTGGAGGAGCGTATAGC	CTCAAGTCTCTCAGTCACCCAAG	RT-PCR
OsSDHA	LOC_Os07g04240	TGGGGTACTGGGAGAATG	GGTGGGAACGACTCAACT	RT-PCR
OsPAL06	LOC_Os02g41680	GGGCAACCCAGTGACCAA	CGATTGCCTCGTCGGTCTT	RT-PCR
OsICS1	LOC_Os09g19734	TATGGTGCTATCCGCTTCGAT	CGAGAACCGAGCTCTCTTCAA	RT-PCR
OsLOX	LOC_Os08g39850	GCATCCCCAACAGCACATC	AATAAAGATTTGGGAGTGACATA	RT-PCR
OsPR1a	LOC_Os07g03710	TCGTATGCTATGCTACGTGTTT	CACTAAGCAAATACGGCTGACA	RT-PCR
OsAOS2	LOC_Os03g12500	CCTCGACGCCAAGAGCTTCC	CGTCCTTGCGAGAGACCAGG	RT-PCR
OsJAmyb	LOC_Os11g45740	GTGATGTCAACAGCAAGAGG	GACTCCGACGTGAACGAATC	RT-PCR
OsPR10	LOC_Os12g36830	CTGTCACCACCATGAAGCTC	TCGATCTTCGTCTCTGTCAC	RT-PCR
Rapid-amplification of cDNA ends				
5'RACE			CCTTTTCGCTTCGGTAGACA	5'RACE
3'RACE		TCGACGGGAATGGTGTAGTAG		3'RACE
subcellular localization,transient protein expression assay				
N9F1	ATGGAGGCCACGGCGGTGAGCATTG		CCCGCTAGGCTTCAGGATTG	transient expression
M9F2	AAGTTACTAGCAATCCACGAGAGAA		AAAGCTAAGCACGGGGTTAATATAG	allele cDNA amplification
BPH9 ATG	ggggacaagtttgtacaaaaaagcaggcttcATGGAGGCCACGGCGGTGAG			subcellular localization
BPH9 101	ggggaccactttgtacaagaaagctgggtcCCCGCTAGGCTTCAGGATTG			subcellular localization